



Raman measurements on gamma irradiated chestnut fruits

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ABSTRACT

Irradiation for food preservation is on use in several countries for different types of foods: fish, meat, vegetables, etc., with its application accepted and regulated by national and international food safety agencies. In this work an irradiation preservation process was applied on chestnut fruits, studying its impact on fruit characteristics. Here, we applied Raman scattering measurements on two varieties of non-irradiated and irradiated chestnut fruits at different irradiation doses (1, 2 and 5 kGy), to identify the main peaks associated with main molecular structures present on chestnut fruits and their behavior (intensity, peak position and full width at half maximum-FWHM) with irradiated doses. The intensity ratios of relevant peaks were used to correlate the obtained results with the dose and/or variety. With the focus of this research centered not only on structural changes after processing but also on the possibility to use this tool to easily distinguish between varieties and/or processed fruits by irradiation. From the results presented in this study, following the appropriate Raman peaks, it was possible to clearly identify the chestnut fruits varieties and also to discriminate irradiated from non-irradiated samples.

From our knowledge, this is the first time that Raman scattering measurements were performed on chestnut fruits submitted to an irradiation preservation process.

1. Introduction

Chestnuts fruits of *Castanea sativa* varieties are cultivated in several regions of the world. In Europe, these fruits are mainly produced in countries such as Portugal, Spain, France, Italy, Greece and Turkey, being the last the main producer of European varieties (FAO, 2021). These fruits have several preservation problems after harvest, due to the presence of fungi and insects (worms and weevils), that could be minimized by post-harvest chemical fumigation, hot-water treatment or other traditional preservation techniques (FAO, 2003). The study of the effects of a post-harvest process for food preservation is generally followed by chemical and physical analysis, to assure that are respected the best standards regarding food safety and quality parameters. Research into food irradiation involves the study of the influence of this novel food process on the integrity of processed products, either in nutritional parameters or in bioactive properties as also the validation of this technology for food preservation, confirming that the main chemical

parameters were not affected by the process (Antonio et al., 2012). In this way, Raman spectroscopy appears here as a fast technique to follow molecular structural changes of food constituents, using less consumables and requiring a simple sample preparation procedure for its analysis (Ilaslan et al., 2015). This technique has been applied with success for analysis of different food compounds (proteins, lipids, carbohydrates, etc.) (Li-Chan, 1996). On fresh food, the main constituent is water, which has not a significant interference on Raman measurements, being for that recommend for *in vivo* and *in situ* studies of biological samples (Li-Chan, 1996). For large molecules, such as polysaccharides, that are also present in chestnut fruits, vibrational spectra may result in overlapping broad bands, making more difficult its identification. However, when under different processing methods (cooking, freezing), these molecules can be more easily identified, thus allowing, using Raman spectroscopy, to monitor changes in the vibrational modes of their structure between different treatments (Li-Chan, 1996).

When food is processed by ionizing radiation, several phenomena

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may occur, mainly the liberation of some compounds, that are attached to other molecules or cell walls, which may protect other constituents from radiation effects (Cabo Verde, 2018). In previous studies, we have reported an increase of phenolic compounds content and antioxidant activity in irradiated chestnut fruits (Antonio et al., 2017). Biochemical analysis of irradiated samples also revealed an increase of glucose and fructose, with the corresponding decrease of sucrose. Generally, radiation breaks the large molecules (starch, sucrose), increasing the quantity of small molecules (raffinose, glucose, fructose), that result from this interaction (Carocho et al., 2012).

Chemical methods give a good quantification for the presence of these different compounds or sub-compounds formed after irradiation (Fig. 1). However, Raman spectroscopy could give a better understanding of molecular structural changes, following the presence or breaking of some bonds, by observing the different vibrational modes on the spectra, with the advantages referred before, a fast method that uses also less consumables (Kizil and Irudayaraj, 2018). Chemical analysis is time consuming, involve reagents that, in some cases, are not environmental friendly, while physical analysis is more direct and allow more easily to assess the changes in food constituents under processing.

Carbohydrates are widespread in natural products. However, since the vibrational modes are less specific than in other biomolecules, the identification of all vibrational modes in the spectra it is not straightforward. In biological food matrixes, where carbohydrates are present, the identification of vibrational modes is more complex, due to the presence of other molecules (Wiercigroch et al., 2017).

Recently, Raman spectroscopy was used to identify the types of sugars in three tropical fruits, that was considered by the authors a tentative approach, "recommending further studies to corroborate the findings" (Balcázar-Zumaeta et al., 2024). Other studies are mainly focused on pure substance or diluted in an aqueous solution (Brizuela et al., 2012; Ilaslan et al., 2015).

Irradiation effects on starch were performed on the pure substance at moderate doses, up to 10 kGy (Kizil et al., 2002) or even higher, up to 100 kGy (De Kerf et al., 2001), that are not appropriate for fresh food products, like chestnut fruits.

Kizil and Irudayaraj applied Raman spectroscopy to discriminate irradiated starch gels at 3, 5 and 10 kGy, following the radiation damages on the C–H and O–H bonds, with the higher percentage of correct discrimination for the higher dose (Kizil and Irudayaraj, 2006). In a previous study, starch from different origin (potato, waxy, wheat, and corn) were irradiated at 3, 5 and 10 kGy, where the authors follow the structural changes by FTIR and Raman spectroscopy, referring the difficulty of exact band assignment due to the complexity of the spectra, and that irradiation of starches under nonaqueous conditions results in subtle changes in the structure, with an acceptable discrimination of irradiated and non-irradiated samples, but not validated for dose discrimination (Kizil et al., 2002).

In a previous work, was analyzed the impact on biological, physico-chemical, nutritional and antioxidant parameters of chestnuts (Antonio

et al., 2012), assessing the effects of gamma irradiation and storage time in energetic value and in major individual nutrients of chestnuts (Fernandes et al., 2011), by chemical analyses.

In this study, using Raman spectroscopy, the effects of gamma radiation post-harvest processing on chestnut fruits were studied. By following the changes on vibrational molecular spectra, we were able to analyze how the molecular compounds of these fruits behave under irradiation at different doses, being the results compared with non-irradiated samples.

Chestnut fruits are mainly starch (amylose and amylopectin), sugars (sucrose, glucose, fructose), with the presence of other minoritarian compounds (Attanasio et al., 2004).

To get an idea of how the process of post-harvest preservation by irradiation, affects each molecular compound or structure present in the fruit, nut fruits from two varieties of *Castanea sativa* (Longal and Judia) were selected and studied by Raman scattering, to study the effect of irradiation on the different molecules in this food matrix, since this interaction depends on the constituents of the food.

Raman measurements for molecular structure characterization after food processing by gamma radiation are scarce in the literature and, from our knowledge, this is the first time that Raman measurements were performed on chestnut fruits submitted to an irradiation preservation process.

2. Materials and methods

2.1. Samples

Chestnut fruits of two *Castanea sativa* varieties (Longal and Judia) were collected in the North of Portugal, in the region of Trás-Os-Montes, Bragança, in November 2019, randomly selected from a bag of about 35 kg and divided in four groups of 1 kg, to be submitted to gamma radiation at different doses.

All samples, control and irradiated, were consistently kept in the same conditions, after harvest in the North of Portugal, when the samples were sent to irradiation facility at CTN, in Lisbon, and when sent for Raman measurements, at the Physics Department, University of Minho, Braga.

2.2. Irradiation

The samples were irradiated at three different doses (1, 3 and 5 kGy), chosen as the typical doses for food processing, using a gamma radiation equipment located at the Campus Tecnológico e Nuclear of Instituto Superior Técnico, Lisbon. This equipment has four ^{60}Co sources and an activity of 74 TBq (January 2020), on a geometry that allowed a good uniformity dose rate. The samples were also rotated during the irradiation and the bags turned upside down at the middle of irradiation time. For dose estimation were used routine dosimeters of Amber Perspex (batch X, Harwell Dosimeters, UK), that were read after irradiation at 603 nm on a UV–Vis spectrophotometer (Shimadzu UV model 1800; Japan), and the dose estimated using a previous calibrated curve for Absorbance versus Dose.

For each bag were used three Amber Perspex routine calibrated dosimeters, on the top, bottom and in the center of the samples. The bags were also rotated up-side down at the middle of irradiation time. The estimated doses are an average of the three measurements. Each dosimeter was also read two times, rotated in the slit of the spectrometer 180°. The calibration curve for routine dosimeters, Absorbance versus Dose, was performed using a standard reference dosimeter, Fricke Ferrous Sulphate, following the preparation procedure described in a previous paper (Fernandes et al., 2011). The equation used to estimate the absorbed dose, D , was $D_{\text{Fricke}} = (278 \Delta A) / ([1 + 0.007(T - 25)][1 + 0.0015(T' - 25)])$, where ΔA is the difference in absorbance measured at 305 nm, between irradiated and non-irradiated solution; T is the solution temperature (°C) during the spectrophotometric measurements and

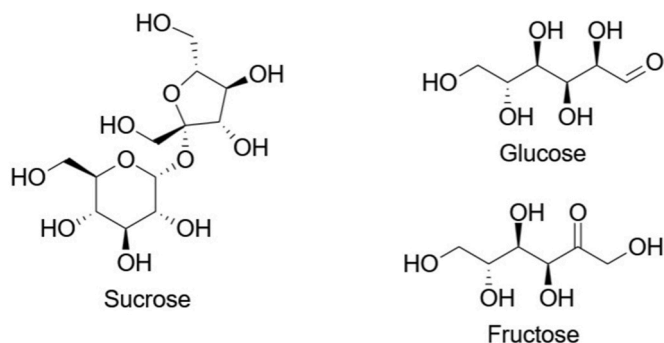


Fig. 1. Sucrose, Glucose and Fructose molecular structures.

T is the irradiation temperature ($^{\circ}\text{C}$) (IAEA, 2002).

The estimated dose with routine dosimeters was obtained following the curve Dose versus Absorbance: D (kGy) = $A + B_1 x + B_2 x^2 + B_3 x^3 + B_4 x^4$, where A , B_1 , B_2 , B_3 , B_4 are the fitting coefficients and “ x ” the specific absorbance.

The samples were irradiated at a dose rate of 1 kGy h^{-1} , with a uniformity dose ratio ($D_{\text{max}}/D_{\text{min}}$) of 1.1, and the estimated doses were $1.0 \pm 0.2 \text{ kGy}$, $2.1 \pm 0.1 \text{ kGy}$ and $5.0 \pm 0.1 \text{ kGy}$ for each sample.

According to previous studies, the optimal dose for chestnut preservation is up to 2 kGy (Antonio et al., 2012; Carocho et al., 2013; Fernandes et al., 2011). Here, to study the impact of irradiation at higher doses on Raman measurements, the samples were irradiated at doses up to 5 kGy .

For simplicity, in the text they are referred as 1, 2, and 5 kGy , respectively, being the control the non-irradiated samples (0 kGy).

2.3. Raman measurements

Raman scattering measurements were carried out at the International Iberian Nanotechnology Laboratory (INL), Braga, on a Alpha300 R confocal Raman microscope (WITec, Germany) using a 532 nm Nd:YAG laser for excitation. The system was operated with an output laser power of 10 mW . The laser beam was focused on the sample by an $\times 50$ lens (Zeiss, Germany); and the spectra were collected at room temperature, with a 600 groove/mm grating using 10 acquisitions with a 2 s acquisition time.

As chestnuts have an outer (exocarp) and inner (endocarp) shells that are non-edible, the Raman study was focused on the edible part of the fruit.

The measurements were taken in three different homogenous points of the fruit, for three fruits randomly selected, with ten measurements for each point in each fruit. For each spectrum, an average of 10 scans were performed at a resolution of 1 cm^{-1} , over the $80\text{--}3200 \text{ cm}^{-1}$ range.

Raman study was focused on the edible part of the fruit, and the measurements were taken in three different homogenous points of the fruit, for three fruits, randomly selected, with ten measurements for each point in each fruit. To obtain the most useful spectral information, multiple scans were performed in 3 different points of the sample by moving the sample on an X–Y stage. The presented Raman spectra is the representative spectrum for each fruit/dose.

3. Results and discussion

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. Raman is a light scattering technique, whereby a molecule scatters incident light from a high intensity laser light source. Then, when a molecular structure is subjected to coherent visible light (laser radiation), they vibrate and emit this coherent radiation in a quantified mode of vibration (phonon) specific to each bond, allowing their identification.

Normally, the Raman spectrum of foods, which typically have long organic molecules, present many vibration modes, very close to each other, often resulting in bands that are the overlap of several modes, making individual association difficult.

In the Raman spectra, the peak position, peak intensity (or area), together with the peak full width at half maximum (FWHM), are important parameters and give information about the molecular structure, whenever there's no superposition with peaks from other molecules with similar structure.

Starch, that is the majority compound present in some foods, including in chestnuts, is a long molecule, formed by the aggregation of small monomers. Starch on chestnuts is composed by amylopectin (about 30%) and amylose (about 70%) (Attanasio et al., 2004), that are long chains of carbon, hydrogen, and oxygen bonds (C – H – O). These fruits contain also other organic molecules such as sucrose and its

monomers, glucose and fructose (Fig. 1). Chemical composition and physical characteristics of the two chestnuts varieties used in this work were object of detailed studies focused on how gamma radiation affected chemical and physical characteristics, at different irradiation doses and at different storage times (Antonio et al., 2012). In this study, Raman measurements of non-irradiated and irradiated samples of the two *Castanea sativa* varieties, were performed as a rapid screening tool, when submitted to a post-harvest preservation process.

In Fig. 2 is shown a representative Raman spectrum of the chestnut fruits, in the range between 80 and 3800 cm^{-1} , where a high number of vibrational modes (22 peaks) are visible.

The Raman spectra of the studied chestnut fruits present information in three main ranges, Range I: around 500 cm^{-1} , Range II: between 800 and 1600 cm^{-1} and Range III: around 3000 cm^{-1} .

Following the molecular structure and the literature, it is possible to assign some of the Raman scattering peaks to bending modes and stretching modes for some of the identified peaks in the different relevant regions of the spectra.

In region I, the 480 cm^{-1} peak mode is assigned to coupling of CCO deformation and CCC deformation. The band at 480 cm^{-1} is an indication of the presence of pyranose ring due to skeletal vibrations mode of the pyranose ring in the glucose unit of starches (Kizil et al. 2002; Flores-Morales et al., 2012). The peak at 524 cm^{-1} is assigned to COC bending modes, for Amylose (Wiercigroch et al., 2017), and the mode at about 850 cm^{-1} to CH₂ twisting mode (Brizuela et al., 2012).

In region II, the peak at 944 cm^{-1} is assigned to the glycosidic bond (Kizil et al., 2002), the 1022 cm^{-1} mode is assigned to CO stretching + CC stretching + OH stretching vibrations (Flores-Morales et al., 2012), and scattering peak at about 1080 cm^{-1} is assigned to stretching modes of CC and CO structures, for Fructose, Glucose and Sucrose. Raman spectra peak at 1130 cm^{-1} is assigned to CO and CC stretching modes, and in plane bending of COH structure, for Glucose and Sucrose (Balcázar-Zumaeta et al., 2024). The modes around 3000 cm^{-1} corresponds to CH_x vibrations and the broad band around 3300 cm^{-1} corresponds to H–O vibrations. The bands in region III, at about 2900 cm^{-1} are associated to CH₂ symmetric stretchings modes (Kizil and Iru-dayaraj, 2006; Brizuela et al., 2012).

By fitting the Raman spectra (using Lorentzian functions), the peak position, intensity and FWHM parameters are obtained for each peak. In Table 1 are presented the peaks present in the Raman spectra and its assignments. These attributions were carried out following the literature (Colthup et al., 2009; Wiercigroch et al., 2017), and allowed identifying the main chemical composition of chestnut fruits.

The spectra, for non-irradiated and irradiated samples, are presented in Fig. 3a) and in Fig. 3b), for Judia and Longal varieties, respectively. The spectra were not normalized, they are shifted vertically for a better visualization. The intensity is expressed in arbitrary units (a.u.).

The results show that, generally, the Raman spectra of both varieties, non-irradiated and irradiated (up to 5 kGy) samples present the same modes, which is the indication that the same components/molecules/bonds/vibrations are present in both varieties, as expected, and that the irradiation process does not impair the fruit quality.

Besides the overall similarity of the spectra presented in Fig. 3, it is seen some dissimilarities, that can be due to the slight differences in the composition of these two varieties (Borges et al., 2008). Indeed, for both varieties, the main differences between non-irradiated and irradiated fruits are seen in the range I and range II, being the most changes related with the intensity of the modes labeled in Fig. 2 (and assigned in Table 1) as 4 (Amylopectin-Amylose), 12 (Glucose) and 15 (Sucrose). However, for both varieties the water band (peak 22, $\sim 3330 \text{ cm}^{-1}$ in the O – H stretch region) show a slight broadening for irradiated samples, as reported by other authors for irradiated starch (Kizil et al., 2002). Food products are complex biological matrixes. When submitted to an irradiation preservation process some components may exert a protective effect on others, directly or by its increase, release, from the biological structure. Generally, when irradiating food, there's an optimum dose

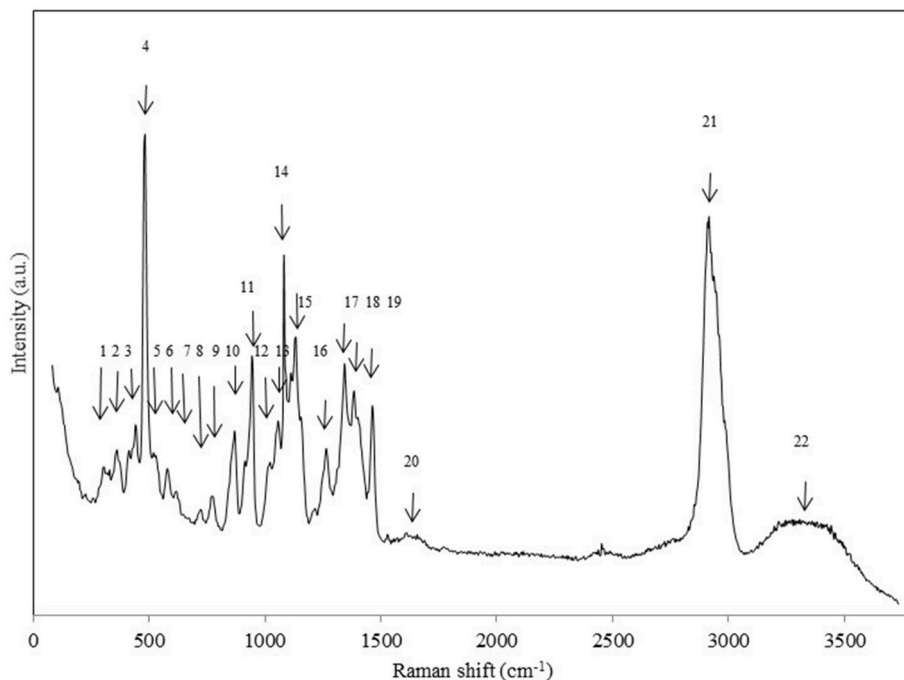


Fig. 2. Representative room temperature Raman spectra of chestnut fruit, namely of Longal variety (L5 – irradiated sample 5 kGy).

Table 1
Raman peak identification for *Castanea sativa* Mill. (Longal and Judia varieties).

Longal	Judia	Literature	Identification
Peak (cm ⁻¹)	Peak (cm ⁻¹)	Peak (cm ⁻¹)	Carbohydrate
1	306	302 (A)	Amylopectin/Amylose
2	362	362 (A, S)	Amylopectin/Amylose; Sucrose
3	446	441 (A, G)	Amylopectin/Amylose; Glucose
4	478	483 (A)	Amylopectin/Amylose
5	524	520 (A, S, F)	Amylopectin/Amylose; Sucrose; Fructose
6	579	584 (S)	Sucrose
7	610	615 (A, F)	Amylopectin/Amylose; Fructose
8	710; 724	714 (A)	Amylopectin/Amylose
9	750; 768	768 (A)	Amylopectin/Amylose
10	856; 869	869 (A, S, F)	Amylopectin/Amylose; Sucrose; Fructose
11	944	944 (A, S)	Amylopectin/Amylose; Sucrose
12	1018	n.d.; 1022 (G)	Glucose
13	1065; 1056	1056 (R, G)	Raffinose; Glucose
14	1091; 1086	1082 (A2, S, F)	Amylose; Sucrose; Fructose
15	1125; 1133	1133 (S)	Sucrose
16	1264	1264 (A, F)	Amylopectin/Amylose; Fructose
17	1344	1344 (S, G, F)	Sucrose; Glucose; Fructose
18	1377; 1381	1385 (R)	Raffinose
19	1460; 1463	1464 (A, R, S, G)	Amylopectin/Amylose; Raffinose; Sucrose; Glucose
20	1661	1657	Carbonyl/Carboxyl band (C=O)
21	2911; 2918	2918 (A, S)	Amylopectin/Amylose; Sucrose
22	3325	3330 (R, G)	Raffinose; Glucose

References: E. Wiercigroch et al. (2017) & Colthup et al. (2009).

n.d. – vibrational mode peak not detected for J0.

(A – Amylopectin/Amylose; S – Sucrose; G – Glucose; F – Fructose; R – Raffinose).

from which the release or degradation of a specific compound reaches its maximum or minimum (Antonio et al., 2017). For that, we opted to follow only some peaks that could give more information about this specific biological food matrix, when submitted to an irradiation preservation process.

In Fig. 4 are presented the peak ratio intensities between the most relevant Raman peaks for non-irradiated and irradiated chestnut fruits, Judia and Longal varieties, namely: peak 1022 (Glucose) and 1133 (Sucrose), compared with peak intensity 480 cm⁻¹ (Amylopectin/Amylose).

This study revealed that the intensity ratio between I₁₀₂₂/I₄₈₀ (Glucose/Amylose) and I₁₁₃₃/I₄₈₀ (Sucrose/Amylose) strongly decrease after irradiation treatment for the Longal variety fruits. However, for Judia variety this decreasing trend of intensity ratios was not evident between non-irradiated and irradiated fruits, indicating that the chemical composition, was conserved after radiation. Based on these results we conclude that Longal variety is more radiation sensitive than Judia variety, that could be attributed to the slight differences in chemical composition for these varieties, namely for sugars content, which is higher in Longal than in Judia varieties (Borges et al., 2008). No relevant effect was observed concerning the radiation dose effect on these intensity ratios, which show only slight variations between doses.

The same Raman study was performed after 3 months of storage: January (for the doses 1, 2, and 5 kGy-results shown in Figs. 3 and 4) and April (for the doses 1 and 2 kGy), which results are shown in Fig. 5. Unfortunately, the chestnuts irradiated at 5 kGy were not in good condition, they were then spoiled. Irradiation at higher doses could compromise the quality of food, namely its texture, that could promote fruit degradation by fungi or by other senescence effects (Antonio et al., 2013; Gandolph et al., 2007).

Previous studies validated the preservation of chestnut fruits by gamma radiation processing at lower doses, up to 1 kGy (Carocho et al., 2013). Here, to study/evaluate how the results obtained by the Raman measurements could be affected, the samples were submitted to a higher dose.

For the non-irradiated fruits, the effects of 3 months (90 days), time storage, is presented in Fig. 5 together with the previous discussed results (0 days) for the Raman peak ratio intensities: Glucose (1022 cm⁻¹)

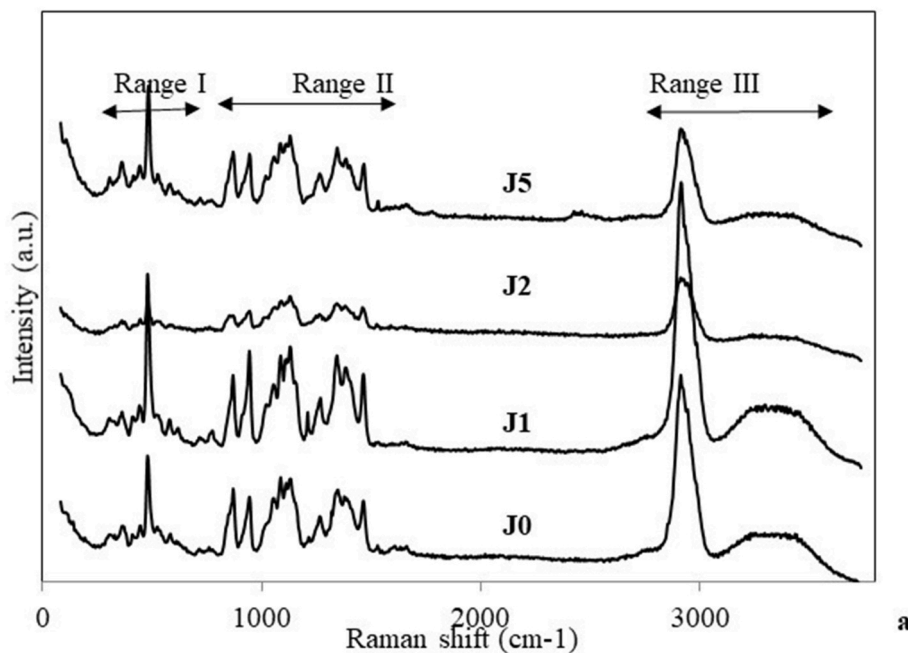


Fig. 3a. Room temperature Raman spectra of Judia variety (J0 – non irradiated; J1, J2, J5 – irradiated samples at 1, 2 and 5 kGy, respectively).

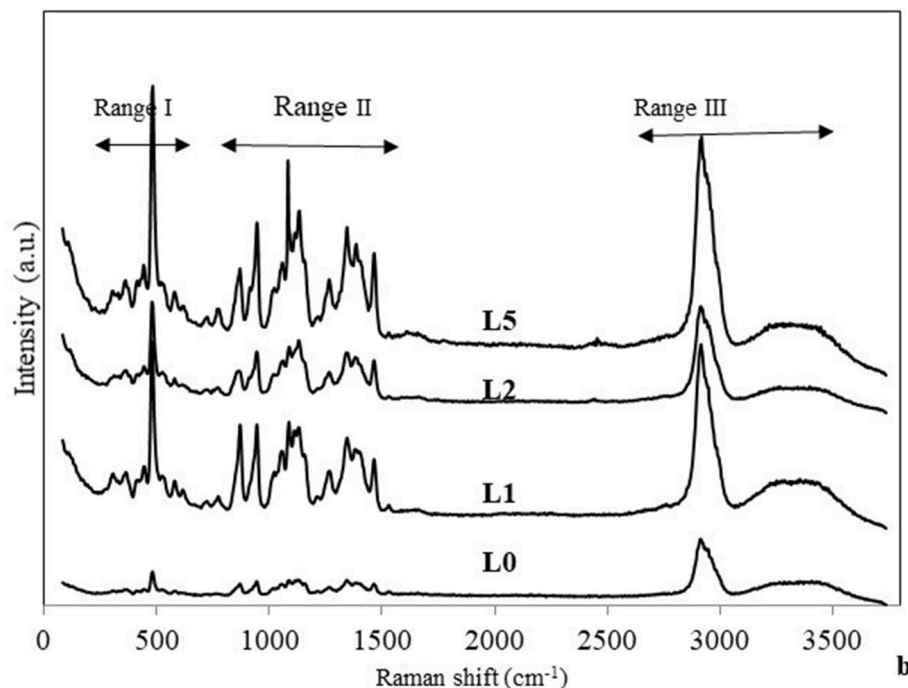


Fig. 3b. Room temperature Raman spectra of Longal variety (L0 – non irradiated; L1, L2, L5 – irradiated samples at 1, 2 and 5 kGy, respectively).

and Sucrose (1133 cm^{-1}) compared with Amylose (480 cm^{-1}), for non-irradiated chestnut fruit varieties, Judia and Longal.

From the results presented in Fig. 5, for non-irradiated fruits, it seems possible to distinguish varieties after harvest (at 0 days), and after storage, at 90 days, for Glucose and Amylose ratios in Longal and Judia varieties). Glucose and Sacaroses ratio intensities for Longal variety are higher, at 0 days and at 90 days, when compared with Judia variety, showing that Longal fruit is sweeter, having higher content of glucose and sucrose than Judia fruit, in agreement with the results obtained by other authors, when compare the chemical composition for these varieties (Borges et al., 2008).

The preservation of chestnut fruits by gamma radiation and storage time effects for both varieties, are presented in Fig. 6 for the selected peak ratios intensities.

Fig. 6 shows the intensity ratio between Glucose and Amylopectin – Amylose ($I_{1022\text{cm}^{-1}}/I_{480\text{cm}^{-1}}$), for Longal (Fig. 6a) and Judia (Fig. 6b) varieties with storage time, for non-irradiated and for irradiated fruits with irradiation dose.

This study reveals that for the non-irradiated fruits, after 3 months storage time, the intensity ratios for Glucose, compared with Amylose, strongly decrease with storage time for both Judia and Longal varieties, being the decrease stronger for Judia variety (Fig. 6). Storage time and

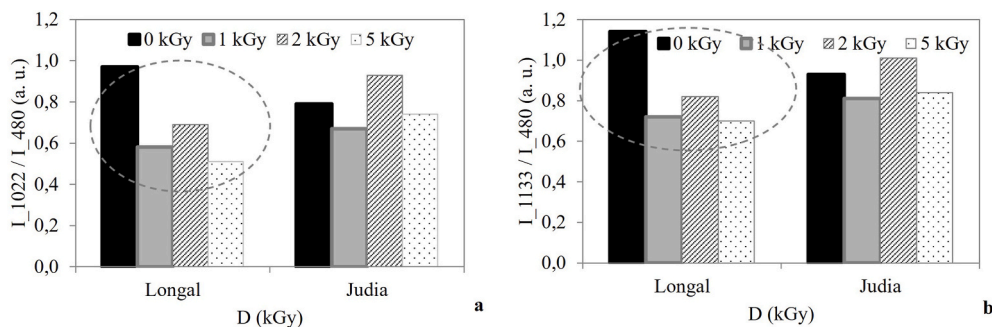


Fig. 4. Behavior of the intensity ratio between the relevant peaks with the irradiation dose. a) peak 12 (Glucose) and 4 (Amylopectin/Amylose); b) peak 15 (Sucrose) and peak 4 (Amylopectin/Amylose).

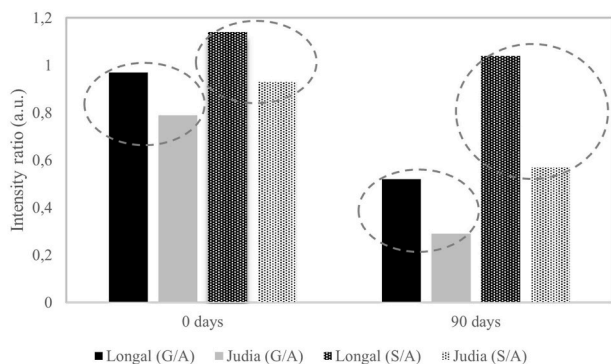


Fig. 5. Behavior of the intensity ratio between relevant Raman peaks at 0 days and 90 days storage time, for Longal and Judia varieties. G-Glucose, S-Sucrose, A-Amylose.

irradiation processing exert simultaneously an alteration of chemical composition of chestnut fruits. Gamma radiation may change molecular structure and/or liberate some compounds from the biological matrix. With storage, this may induce a different behavior of some food components, that could happen in one variety but not in the other, that can be due to the slight differences in the composition of these two varieties (Borges et al., 2008). Moreover, the same effect, *i.e.* a strong decrease in the intensity ratio with storage time is seen for the irradiated fruits, independently of the irradiated dose and variety. Interesting to note that, after 3 months storage time, the intensity ratio is similar in both varieties and doses. Comparing the effect of irradiation dose with the effect of storage time (Fig. 6), we can conclude that the 3 months storage

time effect is more pronounced than irradiation effect, on both varieties, which agrees with previous studies on chemical composition analyses of irradiated chestnut fruits along storage (Fernandes et al., 2011).

4. Conclusion

Radiation processing for post-harvest food preservation is a well-established process, regulated by several national and international authorities, and fully validated as a safe treatment by international food and health organizations, to increase shelf life and/or quarantine phytosanitary treatment. As any other food process: cooking, freezing or roasting; radiation treatment might induce changes in some components, without compromising its quality and safety for human consumption, when restricted to the regulated conditions. In this study, molecular structural changes induced by radiation processing of chestnuts were analyzed by Raman spectroscopy, and here firstly reported. Organic compounds present in these fruits, in which molecular structure are present with C – H and O – H bonds, may be affected by radiation. However, these changes do not necessarily induce the formation of unhealthy compounds. The possible formation of non-healthy compounds, by irradiation processing, were deeply scrutinized by the scientific community, being this food preservation process characterized as safe for human consumers. Here, Raman spectra measurements were validated to discriminate varieties and processed from non-processed fruits by radiation. Where, chestnut fruits of Longal variety are more radiation sensitive than Judia variety.

In the case of disaccharide molecules, such as sucrose, radiation could break glycoside bond, leading to the increase of fructose and glucose, as was previously reported in chemical studies. Food products are complex biological matrixes, when submitted to an irradiation

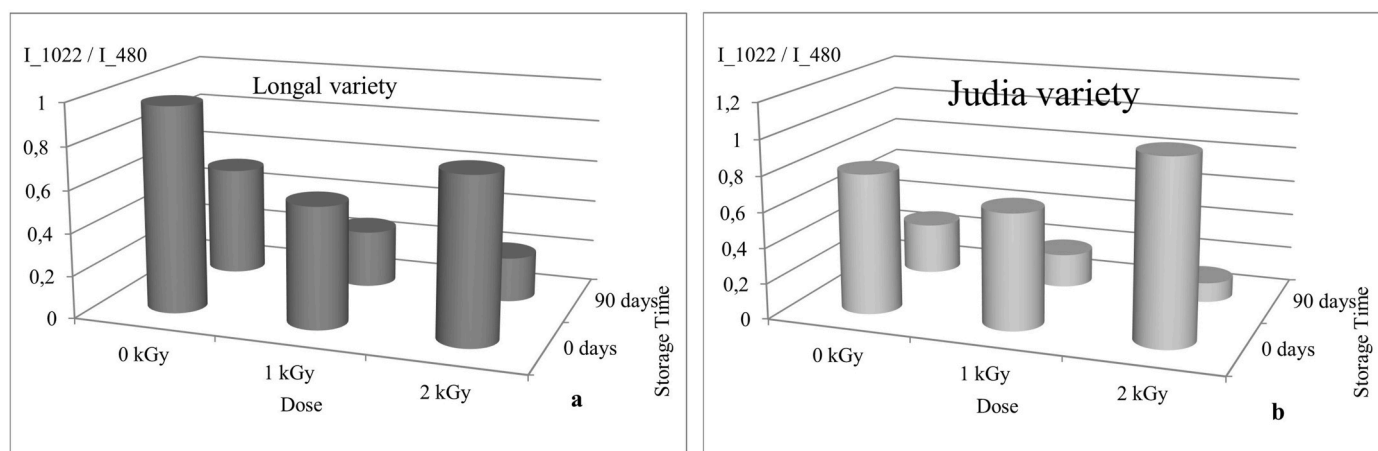


Fig. 6. Behavior of the intensity ratio between peaks 12 (Glucose) and 4 (Amylopectin/Amylose) with the irradiation dose and storage time: a) Longal variety; b) Judia variety.

preservation process some components may exert a protective effect on others, directly or by its increase, release, from the biological structure. Generally, when irradiating food, there's an optimum dose from which the release or degradation of a specific compound reaches its maximum or minimum (Antonio et al., 2017).

Free radicals formed upon radiolysis of water may induce modifications in food structure through very complicated reactions (Kizil and Irudayaraj, 2006; Fernandes et al., 2018). It was possible to also observe that, storage time has a dominant effect than irradiation process, attributed to the hydrolysis of sugars, breaking covalent bonds with the formation of fructose and glucose, becoming the fruits sweeter. And that Raman scattering peak ratios of glucose and sucrose, compared with amylose, could be used to discriminate the varieties, after harvest and after storage. Overall, storage time suggested to introduce more changes than irradiation processing.

From our knowledge, this is the first time that Raman measurements were performed on chestnut fruits submitted to a preservation process by ionizing radiation. To extend its use for food control, generally food agencies require an extended validation by other laboratories, following an inter-comparison study, to fully understand the radiochemistry involved in this preservation process. In fact, this could represent the next step towards the commercial implementation of the methodology proposed in our study, as a rapid screening method for irradiated chestnuts, supporting food safety agencies, while also providing additional information for discerning consumers, who demand higher-quality food products in the market.

CRedit authorship contribution statement

Amílcar L. Antonio: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sandra Cabo Verde:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **M. Fatima Cerqueira:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Data availability

Data will be made available on request.

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